STUDIES ON THE PHENOMENON OF D'HÉRELLE WITH BACILLUS DYSENTERIÆ.

By MARTHA WOLLSTEIN, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

PLATES 35 TO 39.

The phenomenon of d'Hérelle (1) is the expression of a lytic reaction occurring between a bacterium which is inducing an infection in an animal and a substance elaborated in that organism, probably by the leucocytes and other tissue cells, in response to the stimulus of the metabolic products of the invading bacterium. The important element of the reaction is that bacteria exposed to the lytic substance acquire the ability to transfer the lytic property to subsequent generations. The first observations of d'Hérelle were made on stool filtrates from patients convalescent from bacillary dysentery (1). As a result of this and further studies, d'Hérelle (2) concluded that whenever an animal offers resistance to a pathogenic bacterium, a bacteriophage, active for that bacterium, can be isolated from the dejections of the animal. He believes that defense by bacteriophage is a specific immunity reaction. Dumas (3) was able to demonstrate that filtrates from specimens of Paris city water, of Seine water, and of earth, were lytic for colon and dysentery bacilli. He consequently expressed the opinion that the phenomenon is non-specific. The explanation of Dumas' results, as McLeod (4) has stated, is that the specimens he examined were contaminated with excreta infected with colon or dysentery bacilli or both. Specimens from other places may give negative results.

Intestinal Contents.

In an attempt to demonstrate d'Hérelle's phenomenon in human intestinal contents, twenty-three patients at the Babies' Hospital were examined. Six of the children gave a history of gastrointestinal disturbance, while one had been observed throughout an attack of Shiga bacillus dysentery during which the bacillus had been isolated from the stools. Specimens from twenty-two of the twenty-three patients, including the dysentery convalescent, when grown in broth for 24 hours and then filtered through a Berkefeld candle, showed only a negative (non-lytic) reaction between the sterile filtrate and young cultures of *Bacillus dysenteriæ*. One positive reaction was obtained with the filtrate from the feces of an infant who had died of peritonitis due to *Bacillus coli* after intussusception of 3 days duration. In this instance the lytic action was stronger on the colon bacillus than on the dysentery bacillus, and it was possible to transfer the lytic quality to later generations of *Bacillus coli*.

The negative results obtained in the case of the infant who had recovered from an attack of Shiga dysentery may mean that the lytic quality of the intestinal contents is of short duration after such an infection. That the phenomenon of d'Hérelle is not a widespread one among children not ill of gastrointestinal disease is quite evidently demonstrated by these observations.

Dumas' (3) positive lytic reaction with the stools of five of eight adults who gave no history of previous intestinal infection would seem to show that there may be a difference between children and adults in this respect.

Guinea Pig Peritoneal Exudate.

Bordet (5) interpreted the lytic phenomenon of d'Hérelle as due to leucocytic action. He demonstrated its presence in the peritoneal exudate of guinea pigs inoculated with *Bacillus coli* on three occasions at intervals of 5 days. Repeated attempts to reproduce Bordet's results were negative, until a guinea pig was killed after only one intraperitoneal injection; that is, before the peritoneal exudate of the animal had become antilytic by repeated inoculations (Bordet).

The peritoneal exudate is withdrawn by means of sterile capillary tubes from the living animal, and diluted with twice its quantity of sterile broth adjusted to a pH of 8. This is in accordance with Gratia's (6) observation that the lytic action of d'Hérelle proceeds more readily and completely in an alkaline than in an acid medium. In order to rid the guinea pig exudate of fibrin it is well to draw it into a tube containing small sterile beads. The tube containing the defibrinated exudate is sealed and kept for 3 to 30 days to complete the leucocytic action (Bordet). At the end of that time it is centrifuged and the clear supernatant fluid heated for $\frac{1}{2}$ hour at 58°C., after which it is ready to be tested on broth or agar slant cultures. Both must be young, not over 2 to 3 hours old. 1 drop of the fluid to be tested is dropped along one side of an inoculated agar slope, and the tube set upright in the thermostat for observation during the next 48 hours. To broth cultures a larger quantity of the fluid must at first be added, but after one or two generations the lytic fluid may be found to be active in high dilutions.

Growth may be prevented in a broth culture if the lytic agent be added when the tube is inoculated, or young bacilli may be dissolved if the fluid be added after the culture has grown 2 to 4 hours. A broth culture to which the lytic fluid has been added remains or becomes more or less clear for 12 to 24 hours, then it may become somewhat turbid, and clear again after another day. Even the clear tubes are not always sterile, as can be shown by plating. On agar slants the action of the lytic fluid is evidenced by a clear streak along the track of the fluid (Fig. 1). In this clear area there may be no colonies, or there may be a few; that is, some resistant bacilli may remain undissolved and viable. The action of the lytic fluid serves to separate a given culture into sensitive and resistant strains, and by repeating the action on the resistant strains a more and most resistant one can be obtained.

By filtering the clear fluid resulting from the action of the original lytic filtrate or exudate on a broth culture, any quantity of lytic fluid can be obtained, and its action on any number and variety of organisms tested.

The bacteriophage obtained by inoculating a strain of Shiga dysentery bacilli into a guinea pig was found to dissolve its homologous strain, five other Shiga bacillus strains, and two Flexner dysentery bacillus strains. On three strains of Hiss Y bacilli, however, its action was less marked; while three strains of colon bacilli, and one strain

each of typhoid, paratyphoid A and B, mouse typhoid, Morgan bacillus, hog-cholera, and fowl-cholera were not attacked.

Dr. Gratia kindly gave me a small quantity of a lytic fluid which he had prepared by allowing guinea pig exudate, obtained by Professor Bordet in Brussels, to act on a resistant strain of *Bacillus coli*. This bacteriophage proved to be strongly lytic for strains of *Bacillus dysenteriæ* Shiga and less lytic for two strains of Flexner and three strains of Hiss Y dysentery bacilli. The Brussels strain of *Bacillus coli* as well as a strain isolated at the Babies' Hospital some months ago were readily dissolved, but a recently isolated strain of colon bacilli was not affected. *Bacillus typhosus* was acted upon to a slight extent only, but neither a paratyphoid A nor B strain was attacked.

These results showed that the lytic power of the colon phage, made with a resistant *Bacillus coli* strain, is not specific, but that it acts in varying degree on the related strains of the colon-typhoid-dysentery groups of bacilli. The observations of Bordet and Ciuca (7) announcing this fact were published just as our experiments were completed.

One strain each of Friedländer's bacillus, *Bacillus choleræ suis*, *Bacillus avisepticus*, *Bacillus typhi murium*, and Morgan's bacillus was tested with negative results.

With the purpose of studying more fully the effect of the action of a bacteriophage on the dysentery bacillus, a strain of the Shiga type was selected and exposed to the action of the colon phage. A new bacteriophage resulted which was strongly lytic for all strains of dysentery bacilli, for typhoid bacilli, and for the Belgian and American strains of *Bacillus coli*. The paratyphoid strains were still unaffected.

The anti-colon bacillus bacteriophage showed certain group reactions on members of the dysentery-colon-typhoid group. It was most active on the colon strain with which it had been made, less active on a heterologous colon strain; equally active on Shiga dysentery strains, less so on Flexner strains, and still less so on Hiss Y strains; and it was only slightly active on a strain of typhoid bacilli. The Shiga antidysentery bacillus phage, on the other hand, was completely lytic on all strains of Shiga dysentery bacilli and only slightly less so on all strains of Flexner and Hiss Y strains. It was completely lytic for Belgian and American strains of *Bacillus coli* and decidedly lytic for a typhoid bacillus strain. Its group reactions

were stronger than those of the colon bacillus phage. The question naturally presented itself as to whether this denotes a stronger bond between typhoid and dysentery bacilli than between dysentery and colon bacilli, or whether the lytic power of the dysentery bacteriophage was fundamentally stronger for all strains of these three groups. Weight is given to the former supposition by the fact that while the colon phage acted very slightly on the typhoid bacillus, a typhoid phage made from the colon phage by exposure to a strain of *Bacillus typhosus* acted much more strongly on dysentery than on *coli* strains.

Further study of the effect of the action of a lytic fluid upon a strain of Shiga dysentery bacilli is of interest. Not all the bacilli are dissolved, and it follows that those which survive are resistant to the lytic principle. Plates poured from a fluid culture of Shiga dysentery bacilli after 24 or 48 hours contact with a lytic fluid, when the broth culture tube appears to be perfectly clear, show two kinds of colonies (Figs. 2 to 4). One is round, with regular edges, a heaped up or denser center, and rather moist growth. The second kind is very irregular in size and shape, with deep indentations in its thin edges, a depressed center, and very granular. In broth the regular colonies grow rapidly and profusely, the broth becoming turbid. The irregular colonies grow more slowly and fall to the bottom of the tube in granular masses, leaving the broth slightly turbid or almost clear.

A strain of Flexner dysentery bacilli exposed to dysentery bacillus bacteriophage gave rise to regular and irregular colonies exactly comparable to those obtained from the acid or Shiga dysentery strains. Kuttner (8) has shown that the typhoid bacillus develops two kinds of colonies after action of a typhoid bacteriophage.

The experiment presented in Table I was done in order to test the reaction of the bacilli in the two kinds of colonies to the lytic fluid. The table shows that the bacilli in the regular colonies are more resistant to the lytic principle than are those in the irregular colonies.

To ascertain which colonies carry the lytic agent, *i.e.* which are lysogenic, the experiment presented in Table II was done. The irregular colonies are found, then, to be lysogenic and sensitive, while the regular colonies are non-lysogenic and resistant.

Both the regular and the irregular colonies were subcultured on plates for more than 40 generations, and Figs. 5 and 6 show the results. The regular colonies retain their regular outline and characteristics. The irregular colonies, on the other hand, gradually lose their markedly irregular outline and tend to approximate more nearly the regular type of colony, until there are more regular than irregular ones present, and even these are less radically irregular. That is, while the edges of the irregular colonies may still be deeply serrated, the centers are

TABLE I.

Shiga Dysentery Bacilli Plus Shiga Bacteriophage.

Strain.	Medium.	Age.	Result.	
			After 24 hrs.	After 48 hrs.
		hrs.		
Normal, or original............	Broth.	2	Lysis; tube clear.	Same.
Regular.....................	"	2	No lysis; tube turbid.	"
Irregular...................	"	2	Lysis; tube clear.	"

TABLE II.

Normal or Original Strain of Shiga Dysentery Bacillus.

Phage.	Medium.	Age.	Result.
		hrs.	
Normal, original bacillus filtrate............	Broth.	2	Lysis.
Regular colony filtrate....................	"	2	No lysis.
Irregular " "	"	2	Complete lysis.

less hollowed out and gradually become dense (Figs. 7 and 8). The explanation seems to be that the sensitive bacilli die off more rapidly than the resistant ones, which form the regular colonies in later generations. It is a matter of selection.

The two kinds of dysentery bacilli, resistant and sensitive, do not differ from the original culture in their sugar reactions.

An attempt was made to determine whether the acquired lysogenic property is retained permanently or lost in later generations. Working with the original, normal culture as a whole, it was found that

after the seventh generation on agar or in broth the bacilli which had survived contact with a lytic fluid were no longer able to transmit the lysogenic property to other cultures or to dissolve the normal Shiga bacilli. Apparently the sensitive, lysogenic bacilli had all been eliminated in seven generations.

The experiments on this .point were repeated with bacilli from colonies isolated from a normal Shiga culture which had been exposed to dysentery bacteriophage and then plated. The resulting, undissolved bacilli must all be resistant to some degree, but the colonies which grew were of the two distinct types noted above, and were designated sensitive resistant, or irregular, and resistant resistant, or regular. The bacilli from the regular colonies were not dissolved by the dysentery bacteriophage, nor did their broth filtrate dissolve normal Shiga bacilli of the original strain, or the bacilli of the irregular colonies. On bacilli from the regular colonies some lysis resulted. The bacilli from the irregular colonies, on the other hand, were dissolved by the dysentery phage and by their homologous filtrate, which also dissolved normal dysentery bacilli and acted to some extent on the bacilli of the regular type of colony. This was explained by the assumption that the culture of regular, resistant bacilli was not absolutely pure in the sense that some less resistant bacilli were still present. By plating, this was proved to be correct, for both regular and irregular colonies developed from a broth tube of regular bacilli which had been in contact with lytic fluid over night. The regular colonies fished from this plate were again subjected to the action of the strong phage, and plated after 4 days. The resulting colonies were all of the regular type, and were not dissolved by the strong dysentery phage or by filtrates from regular or irregular types of bacilli. It is possible then to obtain a pure, resistant type of dysentery bacilli by repeated elimination of sensitive bacilli from the strain, these resistant bacilli having been present originally.

The sensitive type of dysentery bacillus, after nine generations of broth and plate subculturing, was found to have become more resistant, as shown by the fact that the bacilli of the ninth generation were acted upon less strongly by the antidysenteric phage and by the filtrate from the first generation of irregular bacilli. The filtrate

from the ninth generation was also less lysogenic than the filtrate from the first generation had been.

The sensitive bacilli gradually die out by the action of the lytic substance they carry, and gradually resistant bacilli only are left. The tendency then is for cultures of sensitive bacilli to become more resistant and for resistant bacilli to remain resistant. This is also demonstrated by the colonies on repeated subcultures (Figs. 5 and 6).

The Lytic Reaction under Anaerobic Conditions.

The lytic action proceeds as rapidly and as completely in the absence of oxygen as in its presence. Deep broth cultures with rabbit kidney and sealed with vaseline were inoculated with dysentery bacilli and antidysenteric phage, and growth was prevented as it was in the aerobic controls.

Morphology.

The sensitive strains are composed of short bacilli and many coccoid and round, swollen forms. The resistant strains are made up of regular, equal sized, and evenly staining bacilli, with some larger forms, but few, if any, coccoid or swollen, round forms. Threads are seen in older cultures of the more resistant types.

Virulence.

Rabbits inoculated with minute doses of early broth cultures of the resistant bacilli became paralyzed within 48 hours, and did not recover. Cultures of the sensitive bacilli required five times as great a dose to produce similar results. Apparently the exotoxin of the resistant type of dysentery bacilli is much stronger than that of the sensitive type.

Immunization.

Rabbits were immunized with resistant strains of Shiga dysentery bacilli, with normal strains, and with Shiga bacteriophage. Agglutination tests with the resulting sera brought out the fact that normal, irregular or sensitive, and regular or resistant strains are agglutinated well, the resistant somewhat less so than the sensitive. But a highly resistant strain was not agglutinated even by the sera made with

ordinarily resistant strains. The highly resistant strain killed the animals before they developed immune bodies in their sera.

The animals immunized with antidysenteric phage agglutinated normal dysentery strains in dilution of 1:200, sensitive strains in 1: 1,000, and resistant strains in 1: 100, while the highly resistant strain was not agglutinated.

Evidence which shows that d'Hérelle's phenomenon goes on in the human intestine in a patient with dysentery was recently obtained. At autopsy on an infant 9 months old, who died of bronchopneumonia, a severe pseudomembranous enterocolitis was found. The child had only been under observation for 48 hours, but a history of intestinal symptoms was obtained. By proctoscopic examination on the day before death ulcers were seen in the rectum and a specimen of feces was obtained and plated. On the plates Flexner dysentery bacillus colonies developed with marked, irregular, indented edges. A few small, regular colonies were also noted. Fishings from both kinds of colonies were made, and a sensitive and a resistant strain was obtained. The plates made directly from the rectal specimen were very similar to those obtained in our experiments on the action of the antidysentery lytic fluid on dysentery bacilli. Whether, if a complicating pneumonia had not intervened, the child would have recovered from the dysentery infection or whether the resistant dysentery bacilli were present in sufficient numbers to prevent recovery in spite of the active lysis going on, remains a question.

SUMMARY.

It has been shown that a lytic fluid for dysentery bacilli can be obtained from the peritoneum of the guinea pig by intraperitoneal inoculation of live dysentery bacilli, and that the lytic action of such a fluid is not strictly specific, but that it exerts a group action on the dysentery-colon-typhoid group of bacilli. A lytic fluid with similar effects was obtained from a child dying of Flexner dysentery infection, and an anti-colon bacillus lytic fluid from a child who died of intussusception with colon bacillus peritonitis.

The action of the lytic fluid on the dysentery bacilli, both *in vivo* and *in vitro*, is to divide the culture into sensitive and resistant strains, and the latter can be carried to a degree of very marked, if not complete resistance to lysis. Such resistant strains are not lysogenic, nor are

they agglutinable. The sensitive strains are lysogenic and agglutinable. Varying degrees of sensitive and resistant bacilli exist in a single culture. The sensitive bacilli gradually lose the lysogenic property which they acquired under special conditions, but the very resistant bacilli never acquire that property. It is conceivable that the resistant strains are responsible for the untoward outcome of disease in human beings. Theoretically the administration of lytic fluid should rid the intestinal tract of most of the infecting bacilli, and only if completely resistant bacilli in large numbers remain unacted on is the outcome of the disease a fatal one.

BIBLIOGRAPHY.

1. d'Hérelle, F., *Compt. rend. Acad.*, 1917, clxv, 373.
2. d'Hérelle, F., *Compt. rend. Soc. biol.*, 1921, lxxxiv, 538; *Compt. rend. Acad.*, 1921, clxxii, 99.
3. Dumas, J., *Compt. rend. Soc. biol.*, 1920, lxxxiii, 1314.
4. McLeod, J. W., and Govenlock, P., *Lancet*, 1921, i, 900.
5. Bordet, J., and Ciuca, M., *Compt. rend. Soc. biol.*, 1920, lxxxiii, 1293.
6. Gratia, A., *Compt. rend. Soc. biol.*, 1921, lxxxiv, 275.
7. Bordet, J., and Ciuca, M., *Compt. rend. Soc. biol.*, 1921, lxxxiv, 278.
8. Kuttner, A., *Proc. Soc. Exp. Biol. and Med.*, 1921, xviii, 158.

EXPLANATION OF PLATES.

PLATE 35.

FIG. 1. Effect of the bacteriophage on an agar slope culture of *B. dysenteriæ*.

FIG. 2. Agar plate of a culture of *B. dysenteriæ*.

PLATE 36.

FIG. 3. Regular colonies of *B. dysenteriæ* after the action of the bacteriophage on a normal strain.

FIG. 4. Irregular colonies of *B. dysenteriæ* after the action of the bacteriophage on a normal strain.

PLATE 37.

FIG. 5. Regular colonies of *B. dysenteriæ* after 40 generations of growth on agar plates.

FIG. 6. Irregular colonies of *B. dysenteriæ* after 40 generations of growth on agar plates.

PLATE 38.

FIG. 7. Irregular colonies of *B. dysenteriæ;* third generation; thin edges and hollowed centers.

PLATE 39.

FIG. 8. (a) Regular colonies of *B. dysenteriæ;* 40th generation. (b) Irregular colonies; 40th generation; irregular edge and heaped up center.
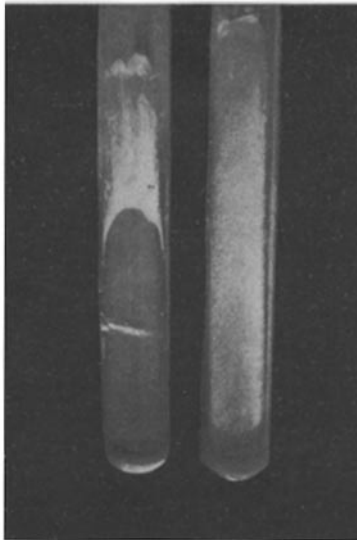
FIG. 1.

FIG. 2.

(Wollstein: Phenomenon of d'Hérelle.)

FIG. 4.

FIG. 3.

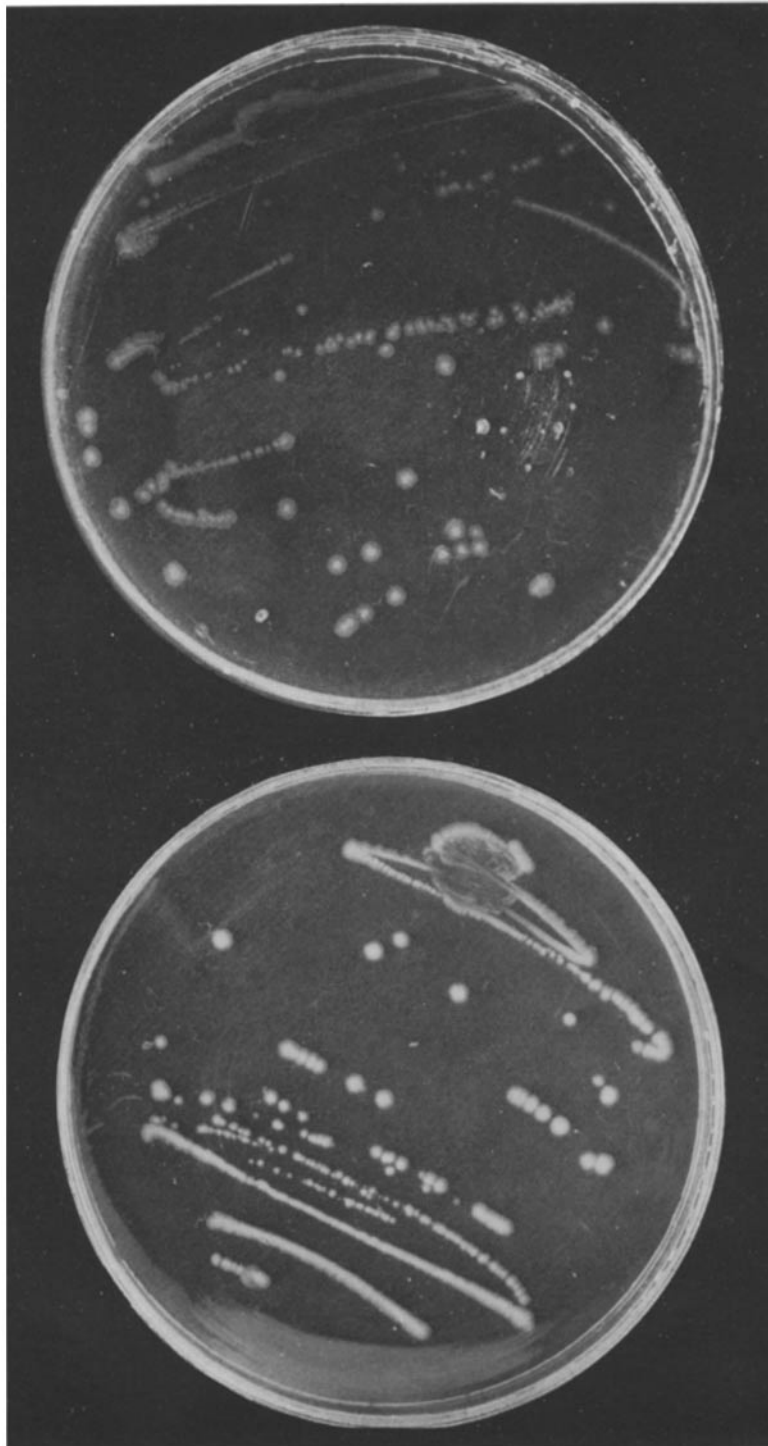(Wollstein: Phenomenon of d'Hérelle.)

FIG. 6.

FIG. 5.

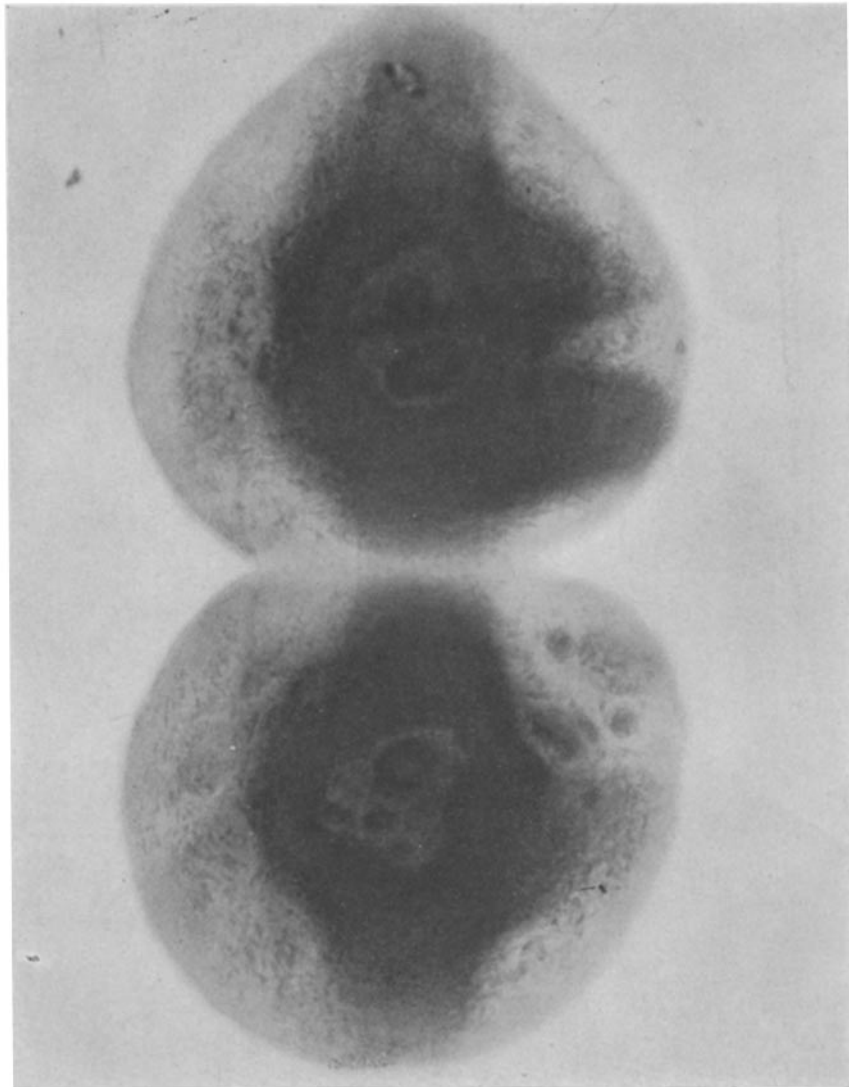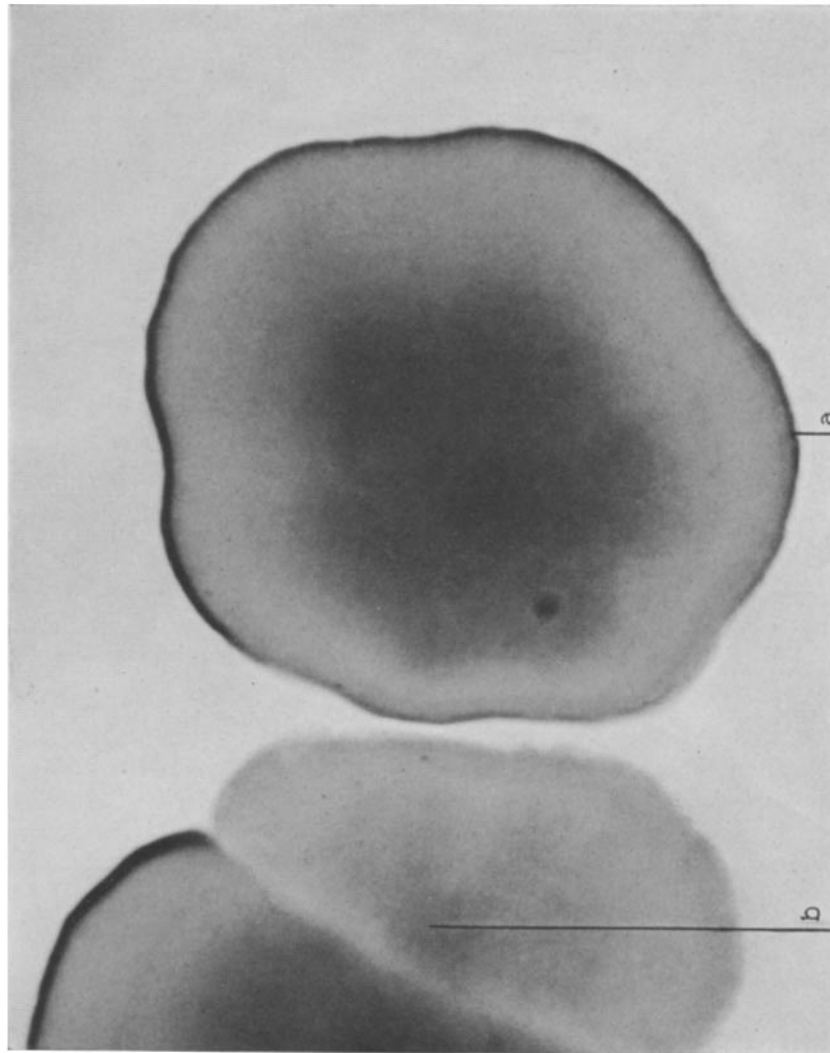(Wollstein: Phenomenon of d'Hérelle.)

Fig. 7.

(Wollstein: Phenomenon of d'Hérelle.)

FIG. 8.

(Wollstein: Phenomenon of d'Hérelle.)